Potentiometric Detection of DNA Molecules Using Field Effect Devices

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1. Introduction

Several types of DNA chips and DNA microarrays have been developed and some of them are used in the field of molecular biology (1). Although most of the current DNA chips and DNA microarrays are based on the fluorescent detection method, amperometric detection methods have been developed in combination with redox reagents (2). We investigated another approach to realize an electrochemical detection for DNA chips and the novel concept of a genetic field effect transistor (FET) is proposed in the present study. Detection of DNA molecules using a genetic FET is in principle based on charge density change at the gate insulator. Fundamental characteristics of a genetic FETs are described.

2. Concept of genetic field effect transistor

The conceptual structure of a genetic FET is shown in Fig. 1. Oligonucleotide probes are immobilized on the surface of the gate insulator. When complementary DNA molecules are contained in a sample solution, they are hybridized with the oligonucleotide probes at the surface of the gate area. Since DNA molecules are negatively charged in an aqueous solution, it is possible to detect them with field effect devices. In the present study, we make use of ionized characteristic of intercalator in an aqueous solution, while it is used as a fluorescent reagent in the conventional methods. Intercalators are ionized and positively charged as shown in Fig. 2, and introduced into double stranded oligonucleotide probes on the gate surface, which leads to increase in the surface charge density. In this way, the signal of hybridization can be enhanced and detected by the use of field effect devices.

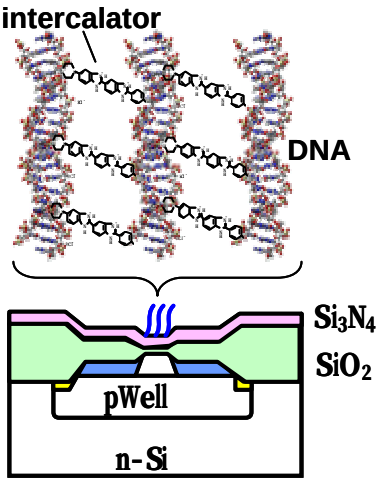

Fig. 1 Concept of genetic FET

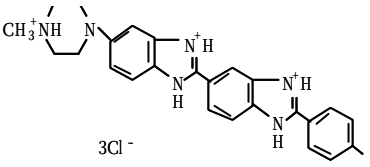

Fig. 2 Chemical structure of typical intercalator

3. Experimental

Oligonucleotide probes were immobilized on the Si_3N_4 surface using 3-(2-aminoethylamino) propyl tri-methoxy silane. After probe deposition, surface groups were blocked with glycine treatment. The detailed procedure for immobilization and base sequences of the oligonucleotide probes used have been reported previously(3). Potentiometric measurements were carried out in a phosphate buffer of pH 6.86 with a

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Ag/AgCl reference electrode.

The threshold voltage (Vt) of the genetic FET was measured with a semiconductor parameter analyzer (Agilent) and Vt shifts were evaluated at each step of surface treatment and reaction with a target DNA and an intercalator.

4. Results and discussion

Typical Vg-Id characteristic of the FET is shown in Fig. 3. The FETs used were n-channel and depletion mode devices. Fig. 4 shows the Vg-Id characteristics with small scales, measured after each step of treatment. The Vg-Id characteristic No. 1 is the same as that shown in Fig. 1. When the surface of the gate was treated with aminosilane and modified with oligonucleotide probes, the Vt was shifted in the positive direction (No.2). This indicates that the negative charges of the oligonucleotide probes were induced at the surface of the gate insulator of the FET. Then, the target DNA molecules were annealed and hybridized at the genetic FETs. A small Vt shift in the positive direction was observed as shown in Fig. 4(No. 3). This also indicates the increase of the negative charge as a result of hybridization at the gate surface.

When an intercalator such as Hoechst 33258 was introduced to the gate surface, the Vt was shifted in the negative direction, which indicated the existence of the positive charges at the gate surface. The larger shift of the Vt was obtained by using the intercalater as compared with hybridization only.

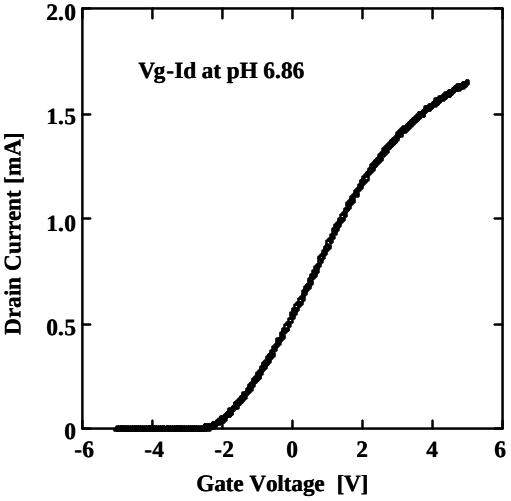

Fig. 3 Typical Vg-Id characteristic of the genetic FET

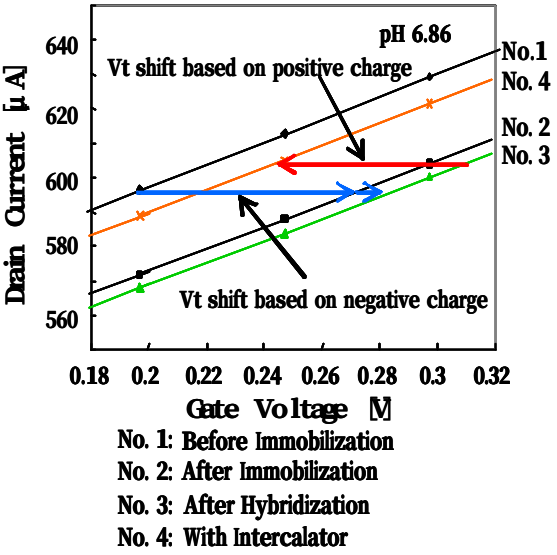

Fig. 4 Threshold voltage shifts of the FET at each step of treatment

5. Conclusions

We developed a new method for detecting DNA molecules using FETs and intercalators. Since most of intercalators have charges, the signal based on the hybridization can be enhanced by introducing intercalator into the double stranded DNA. Preliminary experiments showed that the signal based on hybridization could be enhanced with the use of intercalaor and potentiometric detection of DNA molecules has been successfully demonstrated.

References

(1) D.J. Cutler, M.E. Zwick, M.M. Carrasquillo, C.T. Yohn, et al, Genome Research 11(2001)pp. 1913-1925

(2) S. Takenaka, K. Yamashita, M. Takagi, Y. Uto, H. Kondo, Analytical Chemistry, 72(2000) pp. 1334-1341

(3) T. Kajiyama, Y. Miyahara, L. J. Kricka, P. Wilding, D. J. Graves, S. Surrey and P. Fortina, Genome Research, (2003)pp.467- 475